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Title: Morpho-functional traits and plant response to drought conditions in seedlings of six native species of Ecuadorian Ecosystems.

Authors:

Esteban Chirino^{1,2}, Samantha Ruiz-Yanetti^{3, 6}, Alberto Vilagrosa⁴, Xavier Mera⁵, Manuel Espinoza⁵,
Patricio Lozano⁵

Affiliations:

¹ Investigador Prometeo. Facultad de Recursos Naturales. Escuela Superior Politécnica de Chimborazo.

Panamericana Sur km 1½. Riobamba, Ecuador

² Facultad de Ciencias Agropecuarias. Universidad Laica “Eloy Alfaro” de Manabí. Ciudadela

Universitaria, vía San Mateo s/n. Manta. Manabí. Ecuador

³ Department of Ecology, University of Alicante, Ap. 99 03080 Alicante, Spain

⁴ Mediterranean Center for Environmental Studies (Foundation CEAM). Joint Research Unit University of Alicante – CEAM, Univ. Alicante, PO Box 99, 03080 Alicante, Spain

⁵ Facultad de Recursos Naturales. Escuela Superior Politécnica de Chimborazo. Panamericana Sur km 1½. Riobamba, Ecuador

⁶ Instituto de Ciencias Ambientales y Ecológicas, Universidad de los Andes, 05101 Mérida, Venezuela.

Corresponding author:

Esteban Chirino

Facultad de Ciencias Agropecuarias

Universidad Laica “Eloy Alfaro” de Manabí.

Ciudadela Universitaria, vía San Mateo s/n. Manta. Manabí. Ecuador

Tel. 593 (05) 2 678299 – (05) 2 623740 (ext. 145)

E-mail: esteban.chirino@gmail.com

Highlights

- Drought resistance was assessed in Tropical Andean ecosystem species
- Patterns of biomass allocation, root morphology and gas exchange were studied
- Morpho-functional traits determined the patterns of response to drought
- Drought response of species was independent of their habitats

Abstract

Tropical Andean ecosystems have been identified as very vulnerable to climate change. Changes in climatic conditions, especially drought as envisaged by climate change projections, could affect the establishment of new individuals that are responsible for ensuring the persistence of species and plant communities. The aim was to study the main morpho-functional traits related to water use and drought resistance of native plant species from Ecuadorian ecosystems during imposed drought periods. Seedlings of six native species representative of the Ecuadorian Andes were studied: lower montane evergreen forest (*Alnus acuminata* and *Cedrela montana*), montane cloud forest (*Podocarpus sprucei* and *Aegiphila ferruginea*) and montane dry shrubland (*Schinus molle* and *Caesalpinia spinosa*). Morphological characterisation, a root growth capacity test and stomatal conductance measurements during two controlled drought periods were used to investigate biomass allocation patterns, the root system morphology and gas exchange patterns of species, respectively. The main results indicated that species-specific differences in morpho-functional traits and allocation patterns determined responses to water availability and drought conditions. Low relative growth rate, leaf area ratio and specific leaf area, and high specific root length, were related to drought-resistant species as *P. sprucei* and *C. spinosa*. In contrast, a high or moderate relative growth rate, leaf area and root biomass, but low specific root length as in *A. ferruginea*, *S. molle*, *C. montana*, were related to lower resistance to drought conditions. Despite *A. acuminata* showed high specific root length, it was reported as a species sensitive to drought. Overall, patterns of stomatal conductance, linked to biomass allocation patterns and root system morphology, especially specific root length, seemed to be related to differences in vulnerability to drought in these

species. Drought response was independent of species habitats. Unexpectedly, *P. sprucei*, belonging to montane cloud forest showed higher drought resistance than *S. molle*, belonging to montane dry shrubland.

Keywords. lower montane evergreen forest, montane cloud forest, montane dry shrubland, stomatal conductance, gas exchange

1. Introduction

In the last decade, several studies have indicated the effects of climate change on the distribution of plant species and associated wildlife (González et al., 2010; Bréda and Peiffer, 2014), being particularly vulnerable the mountain forests and relict vegetation (Thuiller et al., 2005; IPCC, 2013). Ecuadorian forests and shrublands are included in Tropical Andean ecosystems, which have been identified as being highly vulnerable to climate change (Urrutia and Vuille, 2009; Buytaert et al., 2011), particularly as a result of changing precipitation regimes and extreme seasonal drought events (Anderson et al., 2011). Drought, flooding and rising temperatures as a result of climate change (IPCC, 2013) can affect the establishment of new individuals guaranteeing the persistence of species and plant communities. On a longer time scale, these changes in climatic conditions could transform the structure of forest ecosystems, or even replace some forest types with others (Mestre and De Cara, 2009). This would, therefore, lead to changes in species composition and diversity. Species less resistant to drought conditions will become more vulnerable to the impact of climate change and will thus be more susceptible to extinction in their current habitats.

Plant responses to drought have been associated with morphological and physiological traits (Hernández et al., 2010; Vilagrosa et al., 2014). Morphological variables are widely used to assess seedling field performance. They are relevant since imbalances in the performance of seedlings, such as changes in the shoot : root ratio (transpiration surface : root absorbing surface) can affect plants' capacity to cope with drought periods (Baquedano and Castillo, 2007) or seedling survival after planting (Grossnickle, 2012).

The variables related to root systems will determine water uptake capacity and contribute to a better seedling water status under drought stress conditions (Chirino et al., 2008; Trubat et al., 2012). Gas exchange variables as stomatal conductance, transpiration or photosynthetic rate have been commonly associated with plant shoot and root morphology and also with xylem resistance to cavitation. All of these variables have been used to assess plant responses to drought conditions (Vilagrosa et al., 2010; Vasques et al., 2013).

In this context, knowledge about the functional traits and patterns of biomass allocation, root structure and rooting habits, and water use efficiency during a drought period can help to improve our capacity to predict the impact of drought on species and communities (Vilagrosa et al., 2014). For this study, we selected six native species from three Ecuadorian ecosystems. *Alnus acuminata* (semi-deciduous tree) and *Cedrela montana* (deciduous tree) which live in lower montane evergreen forest (wet ecosystem) are ecologically relevant for forest regeneration, and are used in pharmaceutical, food and forest industries (Smith, 1960; Alonso-Amelot et al., 2005). *Podocarpus sprucei* and *Aegiphila ferruginea*, both evergreen trees belonging to montane cloud forest (wet ecosystem), are endangered species (IUCN, 2013) and near threatened species (IUCN, 2004), respectively. The other two studied species live in montane dry shrubland (dry ecosystem). They are *Schinus molle* (evergreen tree), which is highly valuable commercially (Brandt et al., 2012; Ibrahim and Haggag, 2013), and *Caesalpinia spinosa* (semi-deciduous tree) which is cultivated as a source of value products (De la Cruz, 2004; López et al., 2011) and medicinal uses (Agapito and Sung, 1998). These six native Ecuadorian species have been extensively studied for their economic, conservation and social importance (Alonso-Amelot et al., 2005; López et al., 2011; Brandt et al., 2012), but have been less studied for their resistance to future environmental stress conditions like drought events (Esperón-Rodríguez and Barradas, 2015).

In a climate change context, studying the drought response of the selected species is singularly important for forest conservation and tropical forest restoration programmes. The hypothesis of this work was that species from dry ecosystems will be more resistant to drought than species from wet ecosystems due to developed functional traits. To test this hypothesis, our objective was to study the main morpho-functional traits related to water use and drought resistance in the selected native plant species of Ecuadorian ecosystems during an imposed drought period. In order to investigate the level of resistance to

drought events, the following issues were analysed: (1) assess the main morphological traits of these species in terms of allocation among roots, shoots and leaf biomass; (2) assess the pattern of response in gas exchange under different soil water content conditions; (3) relate the morphological traits of these species with their gas exchange responses.

2. Material and Methods

2.1. Plant material

Six native species from three vegetation types (montane dry shrubland, montane cloud forest and lower montane evergreen forest) of Ecuadorian ecosystems were selected (Table 1). These species were cultivated for one year in a local nursery. They were grown in polyethylene bags (240 cm³), using a mixture of black peat (60%) and forest soil (40%). The watering regime was 15 mm during the wet season, applied 1 day/week, and 25 mm during the dry season, applied 2 days/week. Before the experiment assessments began, 200 one-year-old seedlings of the six selected species (total: 1.200 seedlings) were transplanted into 3.5-litre pots and cultivated for 3 months (from 28 July to 26 October, 2015) in a standard greenhouse. The standard greenhouse had temperature control, which worked by a system of open-closed windows, but had no automatic air humidity control. The shoot height and basal diameter (mean \pm standard deviation) of seedlings from local nursery was 23.9 ± 8.9 cm and 5.3 ± 1.6 mm respectively. The culture period in the greenhouse and the experimental period were carried out in the nursery of the Faculty of Natural Resources, Polytechnic Higher School of Chimborazo (ESPOCH), located in 1° 39' S and 78° 40' W, at an altitude of 2.726 m a.s.l.

[Place Table 1 here]

A mixture of black peat (40%), forest soil (20%, sandy-loamy texture), river-washed sand (25%), bark of rice grains (10%) and crushed pumice (5%, 3–5 mm grain size) was used as the growing medium for the culture period in the greenhouse. An additional slow-release fertiliser (Basacote Plus, N-P-K: 16-08-12 + Mg + 6 microelements-S) was mixed with the growing medium at a dose of 3 g/L of substrate before transplanting. In the greenhouse, the watering regime was moderate in accordance with seedling growth

and water demand (15 mm in the 2 first months, applied 2 days/week, and 25 mm in the last month, applied 2 days/week), allowing alternation between moist soil (SWC 25–30%) and dry soil (SWC 10–15%). The daily average temperature was 15.4 °C with a mean air humidity of 72.3 % (ESPOCH Weather Station data). The average (7:00–19:00 h) of total solar radiation was 450.5 W.m⁻² (Data from Group of Alternative Energies and Environment of the Faculty of Sciences, CEAA-ESPOCH). The translucent roof of the greenhouse reduced solar radiation by approximately 15% . The culture period in the greenhouse favoured the growth of new roots outside the root plug to colonise the new growing medium in pots. This culture period avoided the effect of the "start size" of the seedlings from the local nursery on seedling morphological characterisation and their response to drought periods.

2.2. Seedling morphology and biomass

Shoot height (H_s , cm) and basal diameter (D , mm) were measured in 30 seedlings per species randomly sampled. Both growth variables were measured at the beginning (28 July, 2015) and the end (26 October, 2015) of the culture period in the greenhouse. The relative growth rate (RGR) was calculated by differences in the average shoot height (H_s) between the end of the culture period in the greenhouse (t_2 , 26 October, 2015) and the initial time (t_1 , 28 July, 2015) using the following equation: $RGR = [\ln(H_{s\ t2}) - \ln(H_{s\ t1})] / (t_2 - t_1)$. A similar procedure for RGR in the basal diameter was carried out.

At the end of the culture period in the greenhouse, morphological characterisation was carried out. Ten seedlings per species were randomly sampled. Seedlings were cut at the cotyledon insertion point and separated into five fractions: leaves, stem, fine roots (diameter < 2 mm), tap root (diameter > 2 mm) and new roots (> 1 cm long), which were growing outside the root plug. The dry weight of each fraction was determined after oven drying at 65 °C for 48 h [leaf dry weight (LDW), stem dry weight (SDW), fine roots dry weight (DW_{FR}), tap root dry weight (DW_{TR}) and new root dry weight (New_DW_R)].

Subsequently, several biomass distribution indices were calculated: shoot dry weight (DW_S), root dry weight (DW_R), seedling dry weight (SM_T), root : shoot ratio (DW_R/DW_S), DW_{FR}/DW_{TR} ratio and DW_R/SM_T ratio. Prior to determining dry weight, leaves were scanned with a professional scanner (Epson Expression 1680 Pro, Seiko Epson Corporation, Nagano, Japan). The obtained images were analysed by the specific image processor WinRhizo software (Regent Instruments, Canada) to obtain the leaf area

(LA, cm²). The specific leaf area (SLA, cm².g⁻¹) was calculated as the ratio between LA (cm²) and LDW (g). The leaf area ratio (LAR, cm².g⁻¹) was calculated between LA (cm²) and SM_T (g).

2.3. Root growth capacity (RGC) test

In order to analyse the selected species' capacity to initiate new root growth to colonise soil, a root growth capacity (RGC) test was run prior to outplanting in bigger containers. Seven seedlings per species were randomly selected and transplanted to 4.8 L PVC tubes (diameter = 11 cm and length = 50 cm) with river-washed sand as substrate. The water regime was 25 mm every 5 days. The RGC test lasted 45 days and was carried out in full sunlight in the nursery at an average temperature of 14.3 °C and a mean air humidity of 71.9% (ESPOCH Weather Station data). At the end of the RGC test, all the new roots (> 1 cm long) that grew outside the root plug were classified according to depth. Previously, PVC tubes were cut lengthwise into two sections to help remove river sand. In 10-cm intervals, from the bottom to the top of PVC tubes, river sand was carefully removed, and new roots were counted, cut and placed inside a polyethylene bag with 10 ml of water, and were finally frozen. Subsequently, and after being defrosted, roots were scanned (400 dpi) in a professional scanner (Epson Expression 1680 Pro). The obtained images were analysed by a specialised software (WinRhizo) to determine root length (RL, cm) and surface area (SA, cm²). Finally, root dry weight (RB, g) was determined after oven drying at 65 °C for 48 h. Specific root length (SRL, m.g⁻¹) was calculated as the ratio between RL (m) and RB (g). The RL/SM_T (m.g⁻¹) ratio and the SA/volume ratio of the PVC tubes (R_{SA}, cm².cm⁻³) were also calculated.

2.4. Daily patterns of stomatal conductance

In order to assess daily patterns of stomatal conductance, a controlled drought experiment was carried out. For this purpose, 30 seedlings per species randomly selected were separated in the greenhouse. Seedlings were watered to field capacity the night before the drought period began, and subsequently, they did not receive rain or watering. Stomatal conductance measurements were taken within three soil water content (SWC) ranges: 30–35% (high), 15–20% (medium) and 5–10% (low). The night before taking the stomatal conductance measurements, five seedlings per species were randomly selected, which fell within the target soil water content range. Soil water content (m³.m⁻³) was measured by a Theta Probe sensor (Delta-

T Devices Ltd., Cambridge, UK). The next day, stomatal conductance (G_s , $\text{mmol.m}^{-2}.\text{s}^{-1}$) was measured in five 1-hour intervals in the daytime (08:00 h, 10:00 h, 12:00 h, 14:00 h and 16:00 h) by a porometer AP4 (Delta-T Devices Ltd., Cambridge, UK). This procedure was repeated for each target soil water content range, under similar ambient conditions (light, air temperature and air humidity) for seedlings. The average temperature was 14.0 °C and the average air humidity was 71.0% (ESPOCH Weather Station data). The daily average total solar radiation was 547.3 W.m^{-2} (CEAA-ESPOCH).

2.5. Stomatal conductance under different soil water content conditions

In order to assess the species response to different drought conditions, another drought period was conducted. In another set, 60 seedlings per species were randomly selected and placed in the greenhouse without rain and without watering. Similarly to the above experiment, seedlings were watered to field capacity the night before the drought period began. In this experiment, stomatal conductance measurements were taken within four target soil water content ranges: 25–30% (high), 20–25% (slightly high), 15–20% (medium), 5–10% (low) and 4–6% (very low). Every 2 or 3 days during the drought period, 10 seedlings per species were randomly selected from the target soil water content range. For this purpose, soil water content ($\text{m}^3.\text{m}^{-3}$) was measured the previous night with a Theta Probe sensor (Delta-T Devices Ltd.). The next morning, stomatal conductance (G_s , $\text{mmol.m}^{-2}.\text{s}^{-1}$) was measured in intervals from 10:30 h to 12:30 h by porometer AP4 (Delta-T Devices Ltd.). This procedure was repeated for the four target soil water content ranges, and under similar ambient conditions (light, air temperature and air humidity) for seedlings. During this drought period, the average temperature was 14.1 °C and average air humidity was 71.5% (ESPOCH Weather Station data). The daily average total solar radiation was 442.2 W.m^{-2} (CEAA-ESPOCH).

2.6. Statistical analysis

Most of statistical analysis were carried out with the SPSS© statistical software, v. 18.0 (SPSS Inc. Chicago, Illinois, USA). Data on seedling morphological characteristics, the RGC test (total values of the root morphology variables), and the stomatal conductance measured in intervals from 10:30 h to 12:30 h under the different soil water content conditions, were compared with an analysis of variance (one-way

ANOVA; factors: species; Tukey's HSD *post hoc* test). General Linear Model Repeat Measures (GLM-ANOVAR) were used to analyse the data of the new root morphology by depth in the RGC test and the stomatal conductance values taken in the daytime. In this analysis, the Greenhouse-Geisser (G-G') value indicates the results of testing the within-subjects effects (differences of species over time), and a P value indicates the results of testing the between-subjects effects (differences between species). We used statistical univariate F Greenhouse-Geisser when the sphericity hypothesis was rejected. Data were transformed when required to assure ANOVA assumptions. Data on soil water content as percentages were transformed by arcsine ($\sqrt{(x\%/100)}$). To better understand the relationship between morpho-functional traits and the response to drought of the studied species, a principal components analysis (PCA) was conducted using the mean values of the variables for each species and the PRIMER-E software (Clarke and Gorley, 2006). Selected morphological and functional variables were SLA, DW_R/DW_S , DW_{FR}/DW_{TR} , SRL, RL/SM_T , LAR, $G_{s_{max}}$ at high SWC (G_s at 12:00 h at SWC: 30–35%), and $G_{s_{max}}$ at low SWC (G_s at 12:00 h at SWC: 4–6%). Most of these variables are the sums of the fractions of biomass or ratios of the morphological variables, which allow several directly measured variables to be analysed. Previously, the relationships between the studied variables were analysed by Pearson correlations.

3. Results

3.1. Morphological traits of seedlings and biomass allocation patterns

Significant differences ($p < 0.01$) were found for the morphological variables among the studied species. Shoot height (H_s) after the growing period in the greenhouse was similar among all the species, but D showed significant differences among species (Table 2). *A. ferruginea* and *A. acuminata* had thicker stems than other species like *C. spinosa* and *P. sprucei*. At the end of the culture period in the greenhouse, *A. acuminata* had a higher H_s_RGR than the other species (Table 2). The lowest values were observed in *C. montana* and *P. sprucei*. *A. acuminata* and *A. ferruginea* had highest D_RGR values, while *C. spinosa* and *P. sprucei* obtained the lowest values (Table 2).

[Place Table 2 here]

Biomass distribution presented significant differences among species. On the one hand, the analysis on aboveground traits indicated that *P. sprucei* had the highest LDW, while *C. montana* and *C. spinosa* had the lowest ones. *A. acuminata*, *A. ferruginea* and *S. molle* had intermediate values (Table 2). SDW showed no significant differences among species. The LA values revealed few differences among all the species, with *C. spinosa* having the lowest values (Table 2). The lowest SLA values were observed in *P. sprucei* and *C. spinosa*, while the highest SLA values were reported for *C. montana* and *A. acuminata* with intermediate values for the other species, *A. ferruginea* and *S. molle* (Table 2). *C. montana* and *A. acuminata* showed highest LAR values, while *C. spinosa* and *P. sprucei* had the lowest ones (Table 2). The analysis on belowground traits yielded that DW_{TR} was higher in *C. spinosa*, *S. molle* and *A. ferruginea* but was lower in *C. montana* and *A. acuminata* (Table 2). DW_{FR} was lower in *C. spinosa* and *S. molle* and higher in *A. acuminata* and *P. sprucei*, with intermediate values for *A. ferruginea* and *C. montana* (Table 2). *A. acuminata*, *P. sprucei*, *C. montana* and *A. ferruginea* were the species with the highest DW_{FR}/DW_{TR} ratio, while *C. spinosa* and *S. molle* had the lowest values for this ratio (Table 2). New_DW_R obtained the highest values in *A. ferruginea* and *C. montana*, and the lowest ones for *C. spinosa* and *P. sprucei* (Table 2). Despite differences being observed in both the root and leaf biomass fractions, no significant differences in DW_R , DW_S and SM_T were found (Table A1, Suppl. Mat.), nor in the DW_R/DW_S ratio (Table 2).

3.2. Root growth capacity of soil colonisation

The morphological traits of the new roots grown outside the root plug (i.e. colonising surrounding soil) were strongly determined by species. The RGC test reported that *S. molle* displayed the maximum rooting depth (Table 3). *A. ferruginea*, *C. montana*, *A. acuminata* and *C. spinosa* showed intermediate values, while *P. sprucei* presented the shallowest root depth, with only one fourth of *S. molle*. *A. ferruginea*, *S. molle* and *C. montana* developed greater biomass of new roots than the other species, and *C. spinosa* had the lowest values (Table 3). RL displayed higher values in *S. molle* and *A. ferruginea*, whereas its lowest values were observed in *C. spinosa*, *P. sprucei* and *C. montana* (Table 3). SA followed a similar trend as RL, with some variations modulated by SRL. Thus *C. spinosa*, *A. acuminata* and *P. sprucei* developed finer roots (SRL values higher than 20 m.g^{-1}), while *C. montana* and *A. ferruginea* developed coarser

roots with values below 9 m.g^{-1} (Table 3). An analysis of root growth by substrate depth showed that *S. molle* quickly developed an extensive root system in both shallow and deep soil layers (about 50 cm, Fig. 1). A similar pattern was observed in *A. ferruginea* and *A. acuminata* with lower deep soil penetration. *P. sprucei* and *C. spinosa* exhibited the least root growth capacity in extension (upper horizons) and in depth (Fig. 1). In fact, *P. sprucei* only showed roots up to a 20-cm depth 45 days after the RGC test began.

[Place Table 3 here]

[Place Fig. 1 here]

3.3. Daily patterns of stomatal conductance

The stomatal conductance curves (G_s), measured on a daily basis under high SWC conditions, showed significant differences in the daytime ($G-G' < 0.01$) and between species ($p < 0.001$; Fig. 2A). Under these SWC conditions, two species groups were found: the first was formed by *A. ferruginea*, *S. molle* and *A. acuminata*, with high G_s rates, especially at midday; and the other group (i.e. *P. sprucei*, *C. montana*, *C. spinosa*) showed moderate rates and a certain degree of stomatal closure at midday. For medium SWC, similar behaviour among species was observed with some stomatal closure at midday in all species (Fig. 2B). This closure was more marked in the species with high G_s rates (i.e. *A. ferruginea*, *S. molle* and *A. acuminata*) than in those with moderate rates (i.e., *P. sprucei* and *C. montana*), in which it was non-existent. With low SWC, no significant differences in G_s among species were observed ($p = 0.842$, Fig. 2C), but were found in temporal dynamics throughout the day (Sphericity assumed = 0.002). All the species displayed a similar behaviour with a drop in G_s at midday, and higher G_s values early in the morning and late in the afternoon when temperature conditions were less stressful.

[Place Fig. 2 here]

3.4. Stomatal conductance dynamics during a drought period

With high SWC (25–30%), *A. acuminata* showed the highest Gs rates ($p < 0.001$) early in the morning ($302.0 \pm 41.6 \text{ mmol m}^{-2} \text{ s}^{-1}$), while the lowest values were observed in *P. sprucei* and *C. montana*, which displayed $86.3 \pm 13.8 \text{ mmol m}^{-2} \text{ s}^{-1}$ and $131.2 \pm 22.9 \text{ mmol m}^{-2} \text{ s}^{-1}$ respectively. *A. ferruginea* ($183.1 \pm 32.9 \text{ mmol m}^{-2} \text{ s}^{-1}$), *S. molle* ($199.9 \pm 34.7 \text{ mmol m}^{-2} \text{ s}^{-1}$) and *C. spinosa* ($191.8 \pm 24.1 \text{ mmol m}^{-2} \text{ s}^{-1}$) gave intermediate values. With prolonged drought, gas exchange rates progressively decreased (Fig. 3). Under slightly high (SWC about 20–25%; Fig. 3A) and medium water availability conditions (i.e. SWC about 10–15%; Fig. 3 B), the maximum *A. acuminata* rates significantly lowered, with values close to 50–60% of their initial Gs values (i.e. SWC 25–30%). On the contrary, the values shown by species like *C. montana*, *P. sprucei*, *A. ferruginea* and *S. molle* came close to 80% of their initial values. The Gs values of *C. spinosa* lowered to about 60–70%. Within the SWC range (10–15%), no significant differences among species were observed (Fig. 3 B). For low water availability (i.e. SWC 5–10%; Fig. 3 C), *A. acuminata* was the species with maximum reduction, about 80% with respect to its initial Gs values, while the other species gave values of around 40–60%. When drought lasted longer (i.e. SWC 4–6%), the Gs values of all the species significantly lowered. Unexpectedly, *P. sprucei* was able to maintain higher Gs rates, with values that came close to 30% of their initial values. Average Gs at low SWC was *C. spinosa* $35.0 \pm 6.9 \text{ mmol m}^{-2} \text{ s}^{-1}$, *A. acuminata* $32.5 \pm 1.8 \text{ mmol m}^{-2} \text{ s}^{-1}$, *P. sprucei* $30.1 \pm 7.9 \text{ mmol m}^{-2} \text{ s}^{-1}$, *S. molle* $21.5 \pm 5.9 \text{ mmol m}^{-2} \text{ s}^{-1}$, *C. montana* $17.4 \pm 5.2 \text{ mmol m}^{-2} \text{ s}^{-1}$, and *A. ferruginea* $6.2 \pm 3.5 \text{ mmol m}^{-2} \text{ s}^{-1}$.

[Place Fig. 3 here]

[Place Fig. 3 here]

3.5. Relationships between morphological traits and stomatal conductance

In order to test the relationship between morphological traits and stomatal conductance, several correlations analyses were done. Under high SWC, $G_{s\max}$ showed a positive correlation with the degree of root soil colonisation (R_{SA} , Fig. 4 A). *A. ferruginea* and *S. molle* obtained higher Gs given their larger SA per volume of explored soil. Under a low SWC, $G_{s\max}$ correlated positively with SRL (Fig. 4 B). Species

such as *C. spinosa* and *P. sprucei* with a high SRL presented high G_s values under these SWC conditions. *A. acuminata* showed a similar result, but its G_s initial was severely reduced. LAR correlated positively with SLA (Fig. 4 C). *P. sprucei* and *C. spinosa* showed a lower LAR (LA per SM_T) and a lower SLA (LA per LDW), which indicates that this species' leaves developed a higher degree of sclerophylly. The SRL variable correlated negatively with the LAR variable (Fig. 4 D). *C. spinosa* and *P. sprucei* presented higher SRL and lower LAR. Variables R_{SA} and RL/SM_T correlated positively with variable D_{RGR} (Fig. A1 Suppl. Mat.), and with maximum rooting depth (Table A2; Suppl. Mat.), respectively. The species with the largest SA per volume of explored soil (i.e. a higher R_{SA}) also had a higher D_{RGR} , and the species with the highest RL/SM_T developed higher rooting depth. Some correlations showed a certain tendency ($P < 0.1$; Table A2; Suppl. Mat.). Variables such as RL/SM_T and D_{RGR} tended to correlate positively with $G_{s_{max}}$ under high SWC ($r = 0.74$, $p < 0.1$, Table A2; Suppl. Mat.), while DW_R/DW_S tended to correlate positively with H_s_{RGR} ($r = 0.76$, $p < 0.1$, Table A2; Suppl. Mat.). In contrast, SRL tended to correlate negatively with SLA ($r = -0.73$, $p < 0.1$, Table A2; Suppl. Mat.).

[Place Fig. 4 here]

The principal component analysis (PCA) of the morpho-functional variables and $G_{s_{max}}$ at high and low SWC resulted in two axes, which explained 83.1% of total variance (Fig. 5). The first axis (PC1) explained 64.1% of total variance and related positively to SRL (0.40), DW_{FR}/DW_{TR} (0.40) and $G_{s_{max}}$ at 4–6% of SWC (0.36), and negatively to RL/SM_T (-0.32), SLA (-0.31), LAR (-0.35) and R_{SA} (-0.40). The second axis (PC2) explained 19.0%, and separated the species in relation to DW_R/DW_S and $G_{s_{max}}$ at 30–35% of SWC. It was positively linked to DW_R/DW_S (0.69) and $G_{s_{max}}$ at high SWC (0.51). The PCA (Fig. 5) divided the species into two groups: on the one hand, *C. spinosa* and *P. sprucei*, compared to a second group represented by *A. ferruginea*, *C. montana* and *S. molle*. *A. acuminata* presents its position in Fig. 5 due to its high $G_{s_{max}}$ at high SWC, similar to *A. ferruginea* and *S. molle* (Fig. 4A), but displayed similar behaviour to *C. spinosa* and *P. sprucei* when SWC decreased (Fig. 4 B). Besides, *A. acuminata* showed high SRL similar to *P. sprucei* and *C. spinosa* (Table 3 and Fig. 4B).

[Place Fig. 5 here]

4. Discussion

4.1. Main morpho-functional characteristics developed by species

Morpho-functional traits varied among species. We found that H_s_RGR , D_RGR , LDW , DW_{TR} , DW_{FR} , New_DW_R , LA , SLA and LAR showed differences among species in aboveground and belowground fractions (Table 2). However, patterns of responses were not the same in the pairs of species for the same vegetation type. High RGR values are related to improved capacity to capture and use resources, and also to seedling development patterns (Galmés et al., 2005). We observed that *A. acuminata*, *A. ferruginea* and *S. molle*, which belong to lower montane evergreen forest, montane cloud forest, and montane dry shrubland, respectively, were the species that simultaneously showed high or moderate H_s_RGR and D_RGR values. We expected that the two species from montane cloud forests, characterised by considerable precipitation and intense shade, would indicate the highest RGR values due to the characteristics of their ecosystem and their specific growth conditions (Pedraza et al., 2003; Ramírez-Marcial et al., 2006). However, exceptions can exist depending on species' growth characteristics and abiotic factors as a result of canopy structure (Gavinet et al., 2015; Granados et al., 2015).

Differences in leaf characteristics and allocation patterns of aboveground biomass were also observed among species. *A. acuminata*, *A. ferruginea*, *S. molle* and *P. sprucei* developed a high LDW and a large LA . In the same context, *A. acuminata*, *S. molle* and *C. montana* showed high values of LAR and SLA . However, a larger LDW was not always related to higher LA values given the differences in SLA among species; e.g. *P. sprucei* had the highest LDW values, but intermediate LA values due to a higher degree of sclerophylly in its leaves (lowest SLA). LA and SLA are key traits that have been related to resistance to drought conditions, and also to the ability to capture resources, such as carbon fixation (Hernández et al., 2010; Valencia et al., 2016).

Root allocation patterns and root morphology play a key role in species response to drought (Chirino et al., 2008). Fine root development has been related to intense soil exploration capacity, and sometimes to high gas exchange rates and water flow throughout the root system (Vilagrosa et al., 2003; Hernández et

al., 2009, 2010). *A. acuminata* was the species with the highest RGR for height and diameter and also showed a higher DW_{FR}/DW_{TR} ratio than the other species. However, this was not a general rule and species like *P. sprucei* or *A. ferruginea* with low or moderate RGR respectively developed a high DW_{FR}/DW_{TR} ratio. Species like *C. spinosa* and *S. molle* developed less fine root biomass, but more coarse roots ($DW_{TR} > 2$ mm). This fact might be related to water limitations in the ecosystem where they live (i.e. montane dry shrubland). In Mediterranean dry ecosystems, species like *Quercus ilex* and *Q. suber* tend to develop a deep root system to reach water reserves in deep soil layers (Tsakalidimi et al., 2005; Chirino et al., 2008). These root types should be coarse so they have the capacity to grow deep in soil and to overcome the mechanical impedance of soils. Deeper soil layers maintain a more stable water content throughout the year (Padilla and Pugnaire, 2007; Chirino et al., 2008), and this species can benefit from these conditions in water shortage periods; e.g., summer drought. In fact, *S. molle* was the species with the maximum rooting depth in the RGC test, with 50 cm over a 45-day period. The other species with a low DW_{FR}/DW_{TR} ratio, *C. spinosa*, had intermediate values.

Differences among species were also revealed by root morphological traits during the RGC test. Some species showed high root development capacity in length, e.g., *S. molle* with a root length over 8 m. Differences in RL or SA were modulated by SRL. We observed that some species developed extremely fine roots with values that exceeded 20 meters per gram of root dry weight (*C. spinosa*, *P. sprucei* and *A. acuminata*), while others developed coarser roots and SRL values below 9 meters per gram of root dry weight (*C. montana* and *A. ferruginea*). SRL is considered a key plant trait as it has been related with other plant functional traits, such as water transport capacity, soil exploration capacity, gas exchange, etc. (Ostonen et al., 2007; Poorter and Ryser, 2015; Roumet et al., 2016). In relation to water dynamics, several studies have found correlations between SRL and the hydraulic conductivity of roots (Rieger and Litvin, 1999; Hernández et al., 2010), which highlights the role of root architecture in plant water status. In fact Trubat et al. (2006, 2012) observed that low nutrient availability promoted thinner roots, higher SRL, and more root junctions. The same authors indicate that these root morphological traits have been related to low water transport capacity and, therefore, to a more moderate use of water under such conditions.

Daily patterns of gas exchange and stomatal sensitivity to water shortage during a drought period also reflected differences among species. The species with high G_s at midday under high SWC (*A. acuminata*, *A. ferruginea* and *S. molle*; Fig. 2A), underwent major stomatal closure at midday with lower water availability (Fig. 2B and C). In contrast, those with moderate G_s (*C. spinosa* and *C. montana*) and low G_s (*P. sprucei*) maintained similar gas exchange patterns in the daytime with lower stomatal closure at midday (Fig. 2A and B). Similar patterns were recorded during the second drought period experiment. Under high SWC conditions (SWC 25–30%), *A. acuminata*, *A. ferruginea* and *S. molle* obtained the highest gas exchange rate, which reflects high capacity for water absorption and transport to leaves. However, they were also the more sensitive species to drought conditions when SWC lowered (Fig. 3D). Species with less tolerance to drought conditions show major reductions in stomatal conductance to maintain a stable leaf water content (McDowell, 2011; Vilagrosa et al., 2010). Unfortunately, this experiment does not have available data on water potential or leaf water content. However, variations in soil water content can be considered a good proxy to estimate water availability in relation to the xylem water potential or leaf water content. Moreover, and in agreement with this study, *A. acuminata* has been previously described as a drought-avoider species, which is very sensitive to water deficit, and avoids water deficit stress by dropping its foliage during drought (Esperón-Rodríguez and Barradas, 2015). On the opposite extreme, we find species like *P. sprucei* with low G_s , with slighter reductions in G_s under drought conditions and relatively high values (i.e. about 30%, Fig. 3D) for SWC of 4–6% compared to the initial G_s values. Those species that present low sensitivity to soil drought conditions can be considered drought-tolerant species, according to Levitt (1980). These species had low gas exchange rates, which allows water to be maintained in soil longer, prolonging water availability for the plant.

Other functional characteristics were related to the root system, e.g., the amount of fine root biomass and SRL, as both traits are related to efficient soil exploration capacity (Hernández et al., 2009; Trubat et al., 2012). These parameters should support exploration and water absorption to maintain gas exchange rates. These previous statements agree with our results. Species as *A. acuminata*, *A. ferruginea*, *S. molle* and *C. montana* showed high RL in upper soil horizons (Fig. 1), high RSA and high gas exchange rates (Fig. 4A). Species that adopt an isohydric strategy should be able to sustain high growth rates and productivity during high water availability periods, but their carbon fixation could sharply drop with strong soil water content reductions (McDowell, 2011). This would be the case for *A. acuminata*, *A. ferruginea* and *C.*

montana. Recent studies have pointed out that this strategy could be at high risk of suffering severe mortality events due to lack of carbon fixation during the common drought periods that have occurred in recent years (McDowell, 2011). In fact extensive plant mortality, associated with species' isohydric behaviour, has been reported elsewhere (Allen et al., 2010; Martínez-Vilalta et al., 2011; García de la Serrana et al., 2015). On the opposite extreme, we find *P. sprucei*, with its high fine root biomass, scleromorphic leaves and low gas exchange rates, but low sensitivity to water shortage, being able to maintain slightly high gas exchange rates under drought conditions. This response pattern reflects some degree of tolerance to drought stress, and is displayed by species with particular adaptations to resist intense drought events, such as high resistance to xylem vulnerability to cavitation (Levitt, 1980; Vilagrosa et al., 2014; Pausas et al., 2016). *S. molle* has been considered a drought-tolerant species (Iponga et al., 2008; Brandt et al., 2014), which agrees with our results. Drought response for *C. spinosa*, *P. sprucei* and *A. ferruginea* has been less studied.

4.2. Morpho-functional traits and drought resistance

The analysis of the functional relationships showed that some morphological and functional variables were closely related, which indicates associations between form and function, with consequences on the drought resistance of species. D_RGR positively correlated with the colonisation of the root system in soil, expressed as R_{SA} (Fig. A1 Suppl. Mat.), and tended to positively correlate with the maximum stomatal conductance rates ($G_{S_{max}}$; Table A2; Suppl. Mat.). $G_{S_{max}}$ (SWC 20–25%) was positively correlated to R_{SA} and showed a tendency to correlate positively with RL/SM_T , and $G_{S_{max}}$ (SWC 4–6%) correlated positively to SRL (Fig. 4 B). This indicates close connections between gas exchange patterns and root growth patterns. In fact root architecture and plant productivity have been related in previous studies (Lynch, 1995; Trubat et al., 2006). In this sense, the results of the present study showed that species with high root growth capacity and high soil colonisation should be able to maintain high G_s rates and higher D_RGR, under optimum SWC.

Other leaf characteristics (e.g. LAR and SLA) correlated positively (Fig. 4C), which indicates that the leaves of the plants with a large LA per dry mass of plant have a lower degree of sclerophylly (high SLA). SRL correlated negatively with LAR (Fig. 4D), i.e. plants with thinner roots (high SRL) had a

small total LA per plant biomass unit (LAR). Indeed the results showed that plants with thicker roots (i.e. lower SRL) produce a larger LA. In line with this, it has been observed that thicker roots are able to conduct more water per transverse section unit to shoots than fine roots (Chirino et al., 2008).

Several studies have related plant functional traits with plant strategy (i.e. rates of resource acquisition and processing rate; Wright et al., 2004; Reich et al., 2014). According to Zhao et al. (2016), *A. acuminata*, *A. ferruginea*, *S. molle* and *C. montana*, species that showed more shallow roots (Fig. 1), and high LAR and SLA (Table 2) could be related with an acquisitive strategy under well-watered conditions (i.e. high water consumption, cheap root tissue investment and rapid returns on that investment in aboveground biomass). In contrast, *C. spinosa* and *P. sprucei* showed lower root biomass, but with a homogeneous vertical root distribution (Fig. 1), and low LAR and SLA (Table 2). This pattern of response would be related to a more conservative strategy (i.e. a water saver strategy, more expensive root tissue investment, and lower aboveground biomass allocation).

The analysis of the relationship between the physiological responses of species and their ecological distribution indicated an unclear association in water use terms. In fact previous studies have reported that species with different water use strategies can co-exist in the same ecosystems due to the morpho-functional traits that determine patterns of responses to water limitations (McDowell et al., 2008; Hernandez et al., 2010; Vilagrosa et al., 2014; Pausas et al., 2016). Drought avoidance responses, such as earlier stomatal closure (Levitt, 1980), observed in several species was not related to a specific type of ecosystems, e.g. species considered avoiders, like *A. acuminata* (Esperón-Rodríguez and Barradas, 2015) or *A. ferruginea*, live in different ecosystems, but with high annual precipitation ($>2.500 \text{ mm} \cdot \text{year}^{-1}$). Other species seem to follow a drought-tolerant strategy due to delayed stomatal closure under lower SWC conditions, such as *P. sprucei* or *C. spinosa*, which live in very different ecosystems like montane cloud forest and montane dry shrubland, respectively, which present a clear difference in the annual precipitation (difference $> 1.500 \text{ mm} \cdot \text{year}^{-1}$).

The PCA analysis also revealed a close relationship among the species that pertain to different ecosystems, and a clear separation among the species that live in similar ones. Indeed a clear separation between *C. spinosa* and *S. molle*, despite them belonging to the same vegetation type (montane dry

shrubland), was observed (Fig. 5). Similarly, a differentiation was found between *P. sprucei* and *A. ferruginea*, both species living in montane cloud forest; and also between *A. acuminata* and *C. montana* which belong to lower montane evergreen forest. The PCA results grouped *A. ferruginea*, *C. montana* and *S. molle*, which were related to high and moderate LAR, SLA RL/SM_T and R_{SA} values. These species showed high gas exchange rates for high SWC, but were much less resistant to drought conditions. Another group, represented by *P. sprucei* and *C. spinosa*, was related to high SRL, but with high and low DW_{FR}/DW_{TR} ratio respectively. These species showed reduced Gs to cope with drought stress, and maintained relatively high values compared to the former group. Similarly to the unclear association between species physiological responses and their ecological distribution, we did not find clear patterns for species' leaf habits. *A. acuminata* also showed high sensitivity to drought conditions, but its position in the PCA results figure (Fig. 5) is due to its high Gs_{max} at high SWC and high SRL. In fact, it showed a marked stomatal closure at midday (Fig. 2) and a sharp drop in Gs_{max} along with *S. molle* and *C. montana* when SWC lowered (Fig. 3).

In summary, the differences in the morpho-functional traits of the studied species determined the response to drought conditions and the ability to maintain slightly high gas exchange rates under suboptimal water availability conditions. Contrary to our hypothesis, the two species that live in the dry ecosystem were not the most drought-resistant. Unexpectedly, *P. sprucei*, an evergreen tree from montane cloud forest has been related to drought-resistant species, jointly with *C. spinosa*, a species belonging to montane dry shrubland. Both species have been related to conservative strategy. Species like *A. acuminata*, *A. ferruginea*, *C. montana* and *S. molle* have been related to less resistance to drought conditions and an acquisitive strategy. According to the estimations made of the potential impact of climate change on the establishment of new individuals of the studied species, the results suggest that the species with functional traits related to a conservative water use, an efficient root-soil colonisation, and moderate allocation patterns in favour of aboveground biomass, would be the least vulnerable species to drought. However, caution is recommended because plant response to drought conditions is the result of a wide array of abiotic and biotic factors that interact in ecosystems.

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References

- Agapito, T., Sung, I., 1998. Fitomedicina: 1100 plantas medicinales. Isabel, Lima.
- Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Hogg, E.H., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecol. Manage.* 259, 660-684.
- Alonso-Amelot, M., Oliveros, A., Arellano, E., 2005. Exhaustive extraction of phenolics and tannins from some sun-exposed forbs and shrubs of the tropical Andes. *Ciencia* 13, 429-439.
- Anderson, E., Marengo, J., Villalba, R., Halloy, S., Young, B., Cordero, D., Gast, F., Jaimes, E., Ruiz, D., 2011. Consequences of climate change for ecosystems and ecosystem services in the tropical Andes. In: Sebastian, K., Herzog, S.K., Martínez, R., Jørgensen, P.M., Tiessen, H. (Eds.), Chapter 1: Climate Change and Biodiversity in the Tropical Andes. San José dos Campos and Paris, pp. 1-18.
- Baquetano, F.J., Castillo, F.J., 2007. Drought tolerance in the Mediterranean species *Quercus coccifera*, *Quercus ilex*, *Pinus halepensis* and *Juniperus phoenicea*. *Photosynthetica* 45, 229-238.

- Brandt, R., Lachmuth, S., Landschulz, C., Haß, F., Hensen, I., 2014. Species-specific responses to environmental stress on germination and juvenile growth of two Bolivian Andean agroforestry species. *New Forests* 45,777-795.
- Brandt, R., Zimmermann, H., Hensen, I., Mariscal, J.C., Rist, S., 2012. Agroforestry species of the Bolivian Andes: an integrated assessment of ecological, economic and socio-cultural plant values. *Agroforest Syst.* 86, 1-16.
- Bréda, N., Peiffer, M., 2014. Vulnerability to forest decline in a context of climate changes: new prospects about an old question in forest ecology. *Ann. For. Sci.* 71, 627-631.
- Buytaert, W., Cuesta-Camacho, F., Tobón, C., 2011. Potential impacts of climate change on the environmental services of humid tropical alpine regions. *Global Ecol. Biogeogr.* 20, 19-33.
- Chirino, E., Vilagrosa, A., Hernández, E.I., Matos, A., Vallejo, V.R., 2008. Effects of a deep container on morpho-functional characteristics and root colonization in *Quercus suber* L. seedlings for reforestation in Mediterranean climate. *Forest Ecol. Manage.* 256, 779-785.
- Clarke, K.R., Gorley, R.N., 2006. *PRIMER v6: User Manual/Tutorial*. PRIMER-E, Plymouth, 192pp.
- De la Cruz, L., 2004. An integral and rational utility of tara (*Caesalpinia spinosa* - *Caesalpinia tinctoria*). *Rev. Inst. Investig. Fac. Minas Metal Cienc. Geogr.* 7, 64-73.
- Esperón-Rodríguez, M., Barradas, V.L., 2015. Ecophysiological vulnerability to climate change: water stress responses in four tree species from the central mountain region of Veracruz, Mexico. *Reg. Environ. Change* 15, 93-108.
- Galmés, J., Cifre, J., Medrano, H., Flexas, J., 2005. Modulation of relative growth rate and its components by water stress in Mediterranean species with different growth forms. *Oecologia* 145, 21-31.
- García de la Serrana, R., Vilagrosa, A., Alloza, J.A., 2015. Pine mortality in southeast Spain after an extreme dry and warm year: interactions among drought stress, carbohydrates and bark beetle attack. *Trees* 29, 1791-1804.
- Gavinet, J., Vilagrosa, A., Chirino, E., Granados, M.E., Vallejo, V., Prévosto, B., 2015. Hardwood seedling establishment below Aleppo pine depends on thinning intensity in two Mediterranean sites. *Ann. For. Sci.* 72, 999-1008.
- González, P., Neilson, R.P., Lenihan, J.M., Drapek, R.J., 2010. Global patterns in the vulnerability of ecosystems to vegetation shifts due to climate change. *Global Ecol. Biogeogr.* 19, 755-768.

- Granados, M.E., Vilagrosa, A., Chirino, E., Vallejo, V.R., 2015. Reforestation with resprouter species to increase diversity and resilience in Mediterranean pine forests. *Forest Ecol. Manage.* 362, 231-240.
- Grossnickle, S., 2012. Why seedlings survive: influence of plant attributes. *New For.* 43, 711-738.
- Hernández, E.I., Vilagrosa, A., Pausas, J.G., Bellot, J., 2010. Morphological traits and water use strategies in seedlings of Mediterranean coexisting species. *Plant Ecol.* 207, 233-244.
- Hernández, E.I., Vilagrosa, A., Luis, V.C., Llorca, M., Chirino, E., Vallejo, V.R., 2009. Root hydraulic conductance, gas exchange and leaf water potential in seedlings of *Pistacia lentiscus* L. and *Quercus suber* L. grown under different fertilization and light regimes. *Environ. Exp. Bot.* 67, 269–276.
- Ibrahim, M.T., Haggag, E. G., 2013. Phenolics from *Schinus molle* stems and their anti-bacterial and anti-microbial activity. *Life Sci. J.* 10, 1931-1937.
- IPCC-Intergovernmental Panel on Climate Change, 2013. Summary for Policymakers. In: Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK and NY.
- Iponga, D.M., Milton, S.J., Richardson, D.M., 2008. Superiority in competition for light: a crucial attribute of the invasive alien tree *Schinus molle* (Peruvian pepper tree) in shaping its impact in semi-arid South African savanna. *J. Arid Environ.* 72, 612-623.
- IUCN, 2004. The IUCN Red List of Threatened Species. <http://www.iucnredlist.org/-details/38135/0>
- IUCN, 2013. The IUCN Red List of Threatened Species. <http://www.iucnredlist.org/-details/42532/0>
- Levitt, J., 1980. Responses of Plants to Environmental Stresses. Volume II. Water, Radiation, Salt, and Other Stresses (2nd Ed.). New York, Academic Press.
- López, A., Oré, R., Miranda, C., Trabucco, J., Orihuela, D., Linares, J., Villafani, Y., Ríos, S., María, S., 2011. Capacidad antioxidante de poblaciones silvestres de “tara” (*Caesalpinia spinosa*) de las localidades de Picoy y Santa Fe (Provincia de Tarma, departamento de Junín). *Scient. Agropecu.* 2, 25-29.
- Lynch, J., 1995. Root architecture and plant productivity. *Plant Physiol.* 109, 7-13.
- Martínez-Vilalta, J., Lloret, F., Breshears, D. D., 2011. Drought-induced forest decline: causes, scope and implications. *Biol. Letters* 8, 689-691.
- McDowell, N., 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiol.* 155, 1051-1059.

- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Yepez, E., 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.* 178, 719-39.
- Mestre, A., De La Cara, J.A., 2009. Impacto del cambio climático en los ecosistemas forestales ibéricos. En: *Predicciones de cambio climático y vegetación. 1er Seminario WCRP-Diversitas (Comités Españoles)*.
- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M.R., Lõhmus, K., Majdi, H., Metcalfe, D., Olsthoorn, A.F.M., Pronk, A., Vanguelova, E., Weih, M., Brunner, I., 2007. Specific root length as an indicator of environmental change. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* 141, 426-442.
- Padilla, F.M., Pugnaire, F.I., 2007. Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Funct. Ecol.* 21, 489–495
- Pausas, J.G., Pratt, R.B., Keeley, J.E., Jacobsen, A.L., Ramirez, A.R., Vilagrosa, A., Paula, S., Kaneakua-Pia, I.N., Davis, S.D., 2016. Towards understanding resprouting at the global scale. *New Phytol.* 209, 945-954.
- Pedraza, R.A., Williams-Linera, G., 2003. Evaluation of native tree species for the rehabilitation of deforested areas in a Mexican cloud forest. *New Forests* 26, 83-99.
- Poorter, H., Ryser, P., 2015. The limits to leaf and root plasticity: what is so special about specific root length? *New Phytol.* 206, 1188-1190.
- Ramírez-Marcial, N., Camacho-Cruz, A., González-Espinosa, M., López-Barrera, F., 2006. Establishment, survival and growth of tree seedlings under successional montane oak forests in Chiapas, Mexico. In: Kappelle, M. (Ed.), *Ecology and Conservation of Neotropical Montane Oak Forests*. Springer Berlin Heidelberg, pp. 177-189.
- Reich, P.B., 2014. The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301.
- Rieger, M., Litvin, P., 1999. Root system hydraulic conductivity in species with contrasting root anatomy. *J. Exp. Bot.* 50, 201–209.
- Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vrignon-Brenas, S., Cao, K., Stokes, A., 2016. Root structure–function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytol.* 210, 815-826.
- Smith, C., 1960. A revision of *Cedrela* (*Meliaceae*). *Fieldiana Bot.* 29, 295-341.

- Thuiller, W., Lavorel, S., Araujo, M.P., Sykes, M.T., Prentice, I.C., 2005. Climate change threats to plant diversity in Europe. *Proceedings National Academy of Sciences USA (PNAS)* 102, 8245-8250.
- Trubat, R., Cortina, J., Vilagrosa, A., 2012. Root architecture and hydraulic conductance in nutrient deprived *Pistacia lentiscus* L. seedlings. *Oecologia* 170, 899–908.
- Trubat, R., Cortina, J., Vilagrosa, A., 2006. Plant morphology and root hydraulics are altered by nutrient deficiency in *Pistacia lentiscus* (L.). *Trees* 20, 334–339.
- Tsakalidimi, M., Zagas, T., Tsitsoni, T., Ganatsas, P., 2005. Root morphology, stem growth and field performance of seedlings of two Mediterranean evergreen oaks species raised in different container types. *Plant Soil* 278, 85–93.
- Urrutia, R., Vuille, M., 2009. Climate change projections for the tropical Andes using a regional climate model: Temperature and precipitation simulations for the end of the 21st century. *J. Geophys. Res.* 114, D02108.
- Valencia, E., Quero, J.L., Maestre, F.T., 2016. Functional leaf and size traits determine the photosynthetic response of ten dryland species to warming. *J. Plant Ecol.* 9, 773-783.
- Vasques, A., Chirino, E., Vilagrosa, A., Vallejo, R., Keizera, J., 2013. The role of seed provenance in the early development of *Arbutus unedo* seedlings under contrasting watering conditions. *J. Exp. Bot.* 96, 11-19.
- Vilagrosa, A., Bellot, J., Vallejo, V.R., Gil-Pelegrín, E., 2003. Cavitation, stomatal conductance, and leaf dieback in seedlings of two co-occurring Mediterranean shrubs during an intense drought. *J. Exp. Bot.* 54, 2015-2024.
- Vilagrosa, A., Hernández, E.I., Luis, V.C., Cochard, H., Pausas, J.G., 2014. Physiological differences explain the co-existence of different regeneration strategies in Mediterranean ecosystems. *New Phytol.* 201, 1277-1288.
- Vilagrosa, A., Morales, F., Abadía, A., Bellot, J., Cochard, H., Gil-Pelegrin, E., 2010. Are symplast tolerance to intense drought conditions and xylem vulnerability to cavitation coordinated? An integrated analysis of photosynthetic, hydraulic and leaf level processes in two Mediterranean drought-resistant species. *Environ. Exp. Bot.* 69, 233-242.
- Wright, I.J., Reich, P.B., Westoby, M. et al., 2004. The worldwide leaf economics spectrum. *Nature* 428, 821–827.
- Zhao, T.T., Arshad Ali, A., Yan, E.R., 2016. The plant economics spectrum is structured by leaf habits and growth forms across subtropical species, *Tree Physiol.* 37, 173-185.

FIGURE CAPTIONS

Figure 1. Comparison of the root length by soil depth between species during the root growth capacity (RGC) test. Results of GLM repeated measures analysis, Mean \pm standard error, N = 7. Test within-factors effects: F' Greenhouse-Geisser (*G-G'*), test between-factors effects: Tukey's HSD *post-hoc* test (*p* value). Abbreviations: *Cedrela montana* (Cm), *Alnus acuminata* (Aa), *Podocarpus sprucei* (Ps), *Aegiphila ferruginea* (Af), *Schinus molle* (Sm) and *Caesalpinia spinosa* (Cs).

Figure 2. Daily patterns of stomatal conductance for three different soil water content conditions during the drought period. Results of GLM repeated measures analysis, Mean \pm standard error, N = 5. Test within-factors effects: F' Greenhouse-Geisser (*G-G'*), test between-factors effects: Tukey's HSD *post-hoc* test (*p* value). We used the statistical Sphericity assumed when the sphericity hypothesis was not rejected. Abbreviations: *Cedrela montana* (Cm), *Alnus acuminata* (Aa), *Podocarpus sprucei* (Ps), *Aegiphila ferruginea* (Af), *Schinus molle* (Sm) and *Caesalpinia spinosa* (Cs).

Figure 3. Remaining stomatal conductance (%) during the drought period referred to maximum data indicated in subsection 3.4 (first paragraph). Soil water content conditions in each period were A: SWC (20–25%), B: SWC (10–15%), C: SWC (5–10%) D: SWC (4–6%). Results of one-way ANOVA were significant for A, C and D at $P < 0.05$. Species were pooled by groups according to Tukey's HSD *post-hoc* test, with species having the same letter being not significantly different from each other. Mean \pm SE for N = 10. Abbreviations: *Cedrela montana* (Cm), *Alnus acuminata* (Aa), *Podocarpus sprucei* (Ps), *Aegiphila ferruginea* (Af), *Schinus molle* (Sm) and *Caesalpinia spinosa* (Cs).

Figure 4. Correlations between morphological and physiological parameters with Pearson's correlation coefficient (*r*) and significance levels (*, $p < 0.05$; **, $p < 0.01$). Abbreviations: maximum stomatal conductance ($G_{s_{max}}$), ratio surface area per volume PVC tube (R_{SA}), specific root length (SRL), leaf area ratio (LAR), specific leaf area (SLA), *Cedrela montana* (Cm), *Alnus acuminata* (Aa), *Podocarpus sprucei* (Ps), *Aegiphila ferruginea* (Af), *Schinus molle* (Sm) and *Caesalpinia spinosa* (Cs). $G_{s_{max}}$ Fig. A,

corresponds to G_s at high SWC (values at 12:00 h at SWC: 30–35%), and $G_{s_{max}}$ Fig. B, corresponds to G_s at low SWC (values at 12:00 h at SWC: 4–6%).

Fig. 5 Results of Principal Components Analysis (PCA). Distribution of variables (left and bottom axis) and the studied species (right and top axis) as a function of principal components 1 and 2 resulting from multivariate analysis among morpho-functional traits and gas exchange variable. Abbreviations: root : shoot ratio (DW_R/DW_S), tap root dry weight (DW_{TR} , > 2 mm), fine roots dry weight (DW_{FR} , < 2 mm), seedling dry weight (SM_T), leaf area ratio (LAR), specific leaf area (SLA), root length (RL), Specific root length (SRL), surface area per volume of soil explored (R_{SA}), *Cedrela montana* (Cm), *Alnus acuminata* (Aa), *Podocarpus sprucei* (Ps), *Aegiphila ferruginea* (Af), *Schinus molle* (Sm) and *Caesalpinia spinosa* (Cs).). $G_{s_{max}}$ at 30–35% corresponds to G_s value at 12:00 h at SWC 30–35%, and $G_{s_{max}}$ at 4–6% corresponds to G_s value at 12:00 h at SWC 4–6%. Different vegetation types are indicated by different symbols (circle: montane evergreen forest, triangle: montane cloud forest, square: montane dry shrubland).

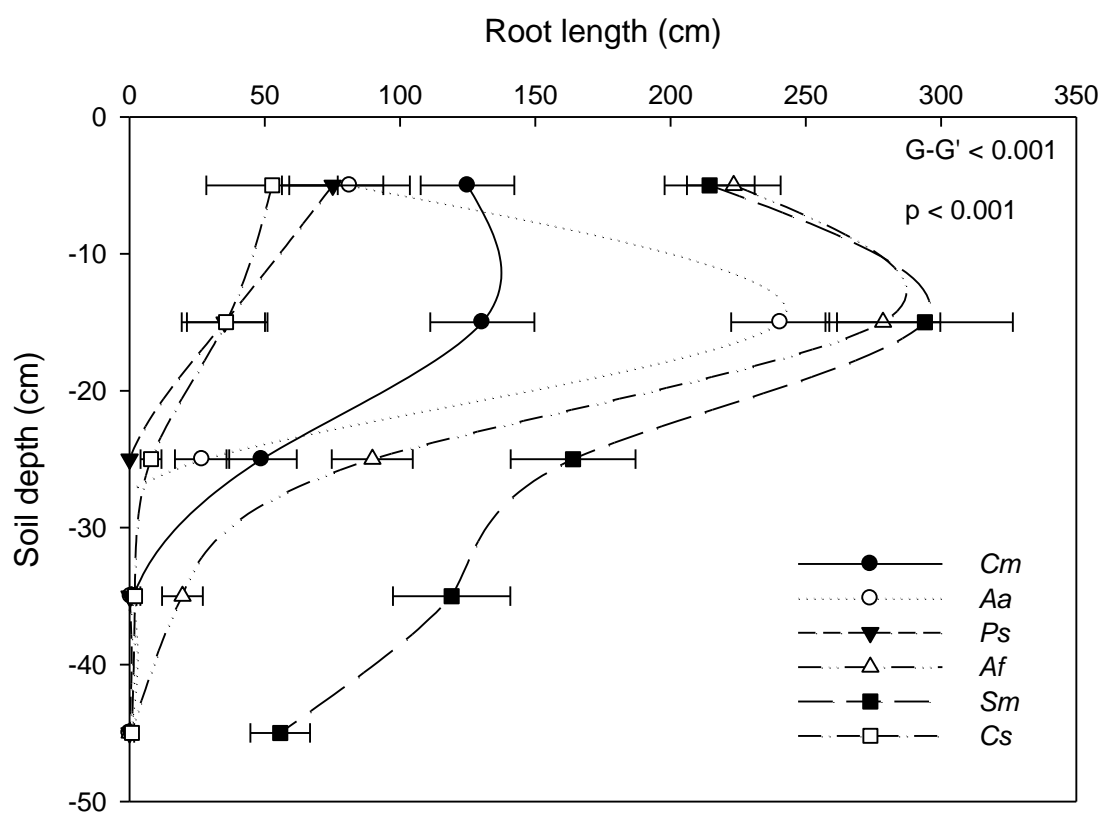


Fig. 1

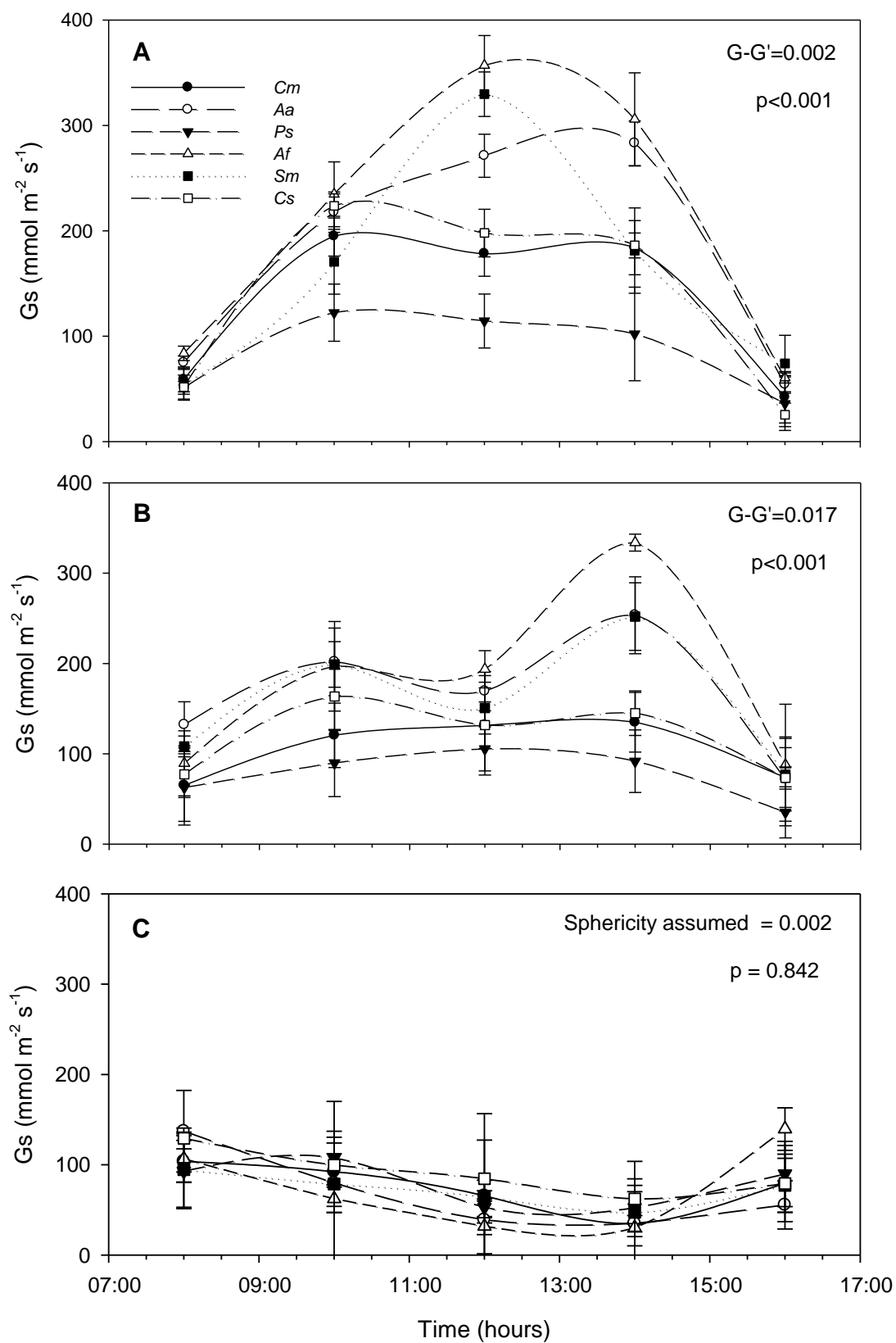


Fig. 2

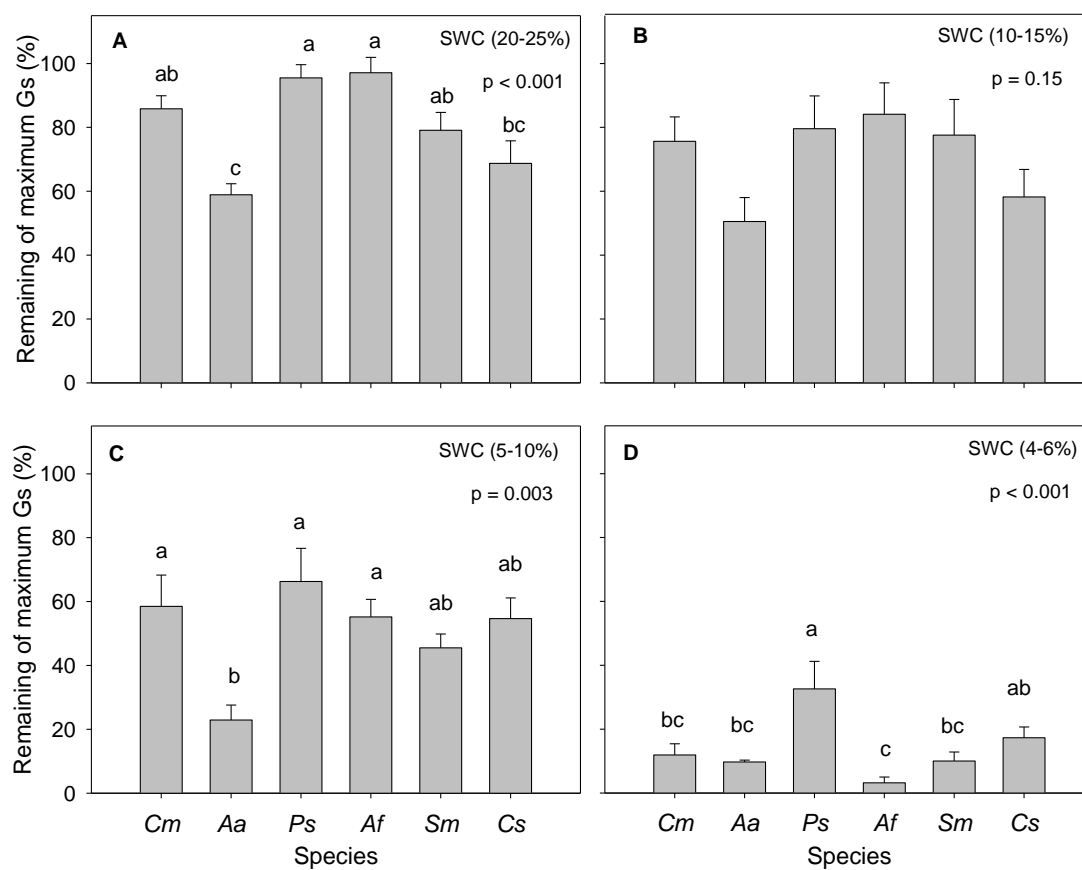


Fig. 3

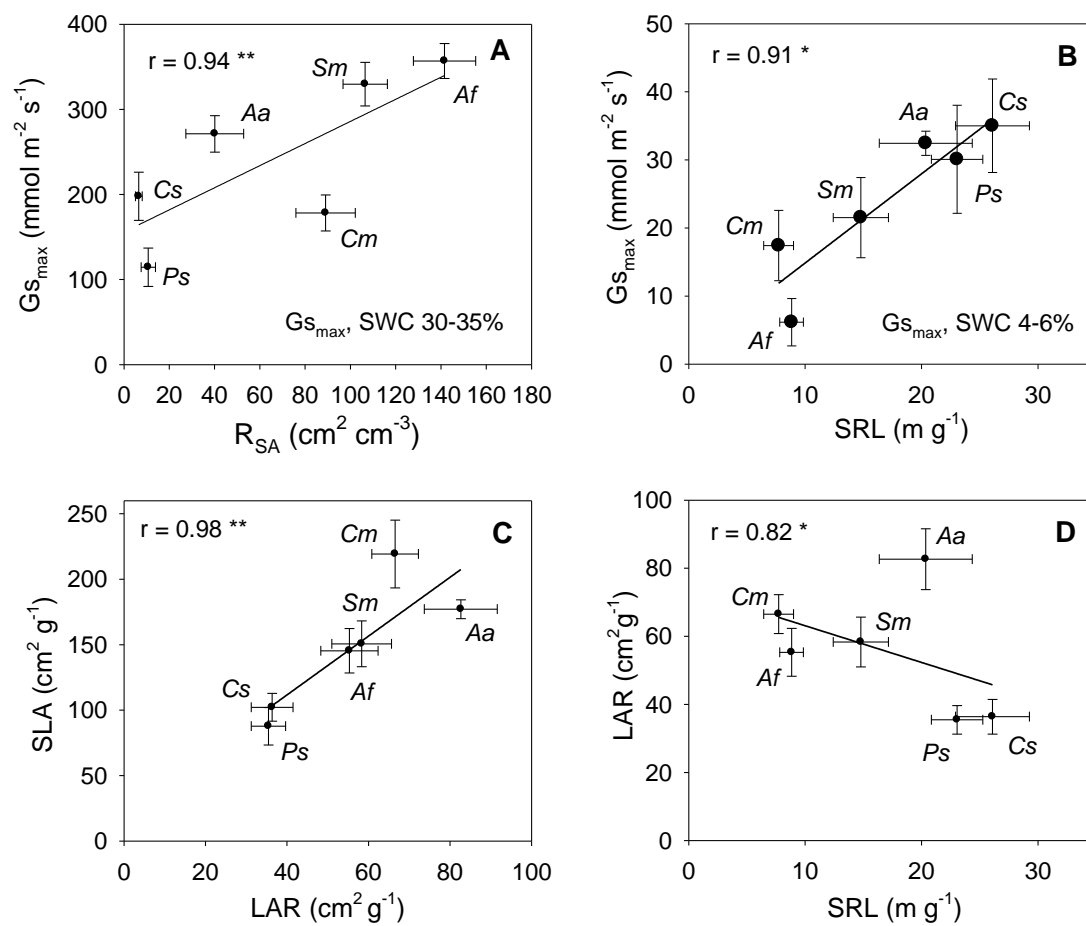


Fig. 4

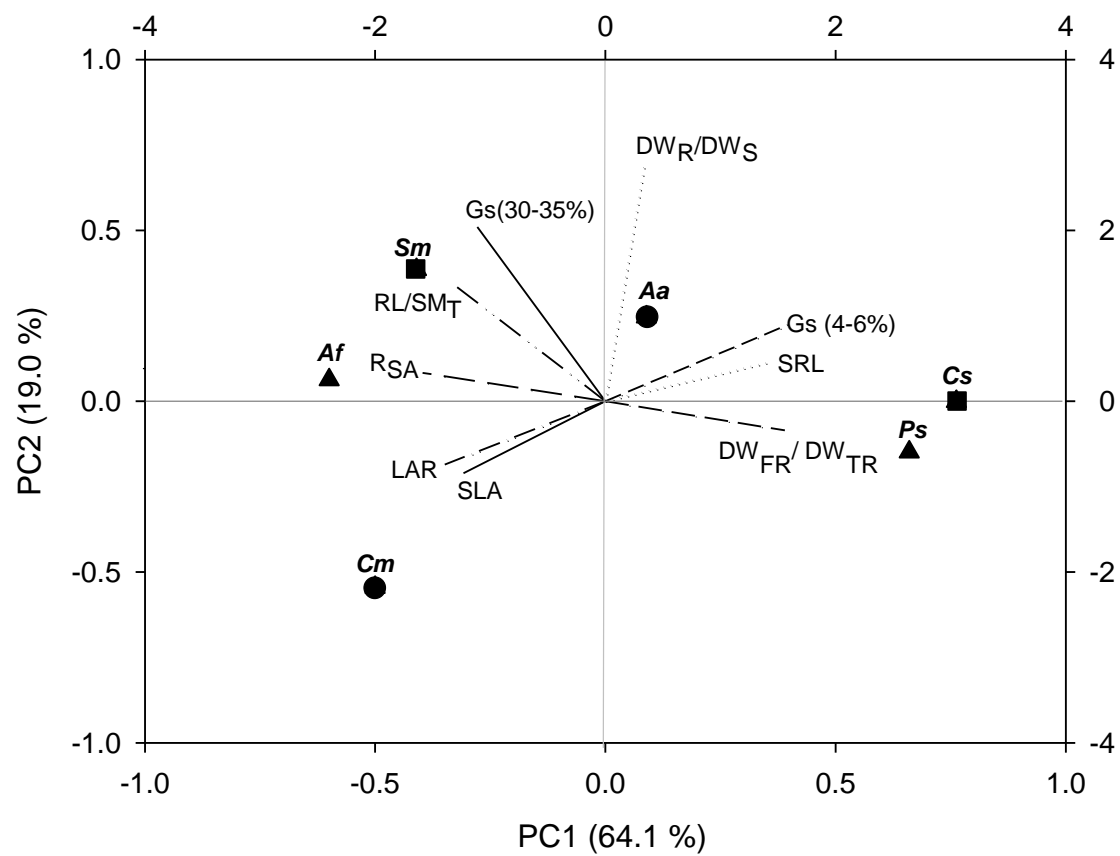


Fig. 5

TABLES

Table 1. Studied native species of Ecuadorian Ecosystems. Description, importance and economical value

Vegetation type	Scientific name	Species code	Description	Importance and economical value
Montane dry shrubland (Dry ecosystem)	<i>Caesalpinia spinosa</i> (Molina) Kuntze	Cs	Small leguminous tree or thorny shrub	-Seed and fruit as source of dyes for textiles and tannins for leather industry -Food industry (gum) -Medicinal value as antioxidant, antiallergic, antimutagenic, anticarcinogenic and antiinflammatory
	<i>Schinus molle</i> L.	Sm	Evergreen tree	-Medicinal purposes as repellent, fumigant, as analgesic and as an antibacterial -Commercial value as ornamental tree, flavoring, pepper and as essential oil
Montane cloud forest (Wet ecosystem)	<i>Podocarpus sprucei</i> Parl.	Ps	Evergreen tree	-High quality wood used for furniture and light construction
	<i>Aegiphila ferruginea</i> Hayek & Spruce	Af	Broad-leaved evergreen tree	-Used in ecosystem restoration
Lower montane evergreen forest (Wet ecosystem)	<i>Alnus acuminata</i> Kunth	Aa	Semi-deciduous tree, broad-leaved	-Good quality wood -Medical attributes
	<i>Cedrela montana</i> Moritz ex Turcz.	Cm	Deciduous tree	- High economic value due to the high durability and hardness of its wood

Table 2. Seedlings morphological characteristics at the end of the culture period in the greenhouse. Results from one-way ANOVA (Mean \pm standard error, N = 10; Tukey's HSD *post-hoc* test). The p values in bold indicate significant differences at $p < 0.05$. Abbreviations: shoot height (H_s), basal diameter (D), relative growth rate in height (H_s_RGR), relative growth rate in basal diameter (D_RGR), leaf dry weight (LDW), stem dry weight (SDW), shoot dry weight (DW_s), leaf area (LA), leaf area ratio (LAR), specific leaf area (SLA), tap root dry weight (DW_{TR} , > 2 mm), fine roots dry weight (DW_{FR} , < 2 mm), root dry weight (DW_R), seedling dry weight (SM_T), new root dry weight grown outside of the root plug (new_DW_R), root : shoot ratio (DW_R/DW_s), *Cedrela montana* (Cm), *Alnus acuminata* (Aa), *Podocarpus sprucei* (Ps), *Aegiphila ferruginea* (Af), *Schinus molle* (Sm) and *Caesalpinia spinosa* (Cs).

Morphological		Species						P value
characteristics		Cm	Aa	Ps	Af	Sm	Cs	
H_s	cm	29.8 ± 2.5	32.1 ± 7.9	37.574 ± 1.7	23.7 ± 2.5	27.4 ± 1.6	24.6 ± 3.9	0.154
D	mm	$5.9 \pm 0.4bc$	$7.5 \pm 0.5ab$	$5.4 \pm 0.1c$	$7.9 \pm 0.5a$	$6.2 \pm 0.4abc$	$5.4 \pm 0.3c$	0.001
H_s_RGR	year ⁻¹	$0.11 \pm 0.01c$	$0.34 \pm 0.03a$	$0.07 \pm 0.01c$	$0.17 \pm 0.02b$	$0.16 \pm 0.01b$	$0.21 \pm 0.02b$	0.001
D_RGR	year ⁻¹	$0.13 \pm 0.03b$	$0.21 \pm 0.04a$	$0.04 \pm 0.01c$	$0.18 \pm 0.04ab$	$0.14 \pm 0.03b$	$0.05 \pm 0.01c$	0.001
LDW	g	$4.87 \pm 0.78b$	$5.58 \pm 0.56ab$	$8.17 \pm 1.00a$	$6.53 \pm 0.88 ab$	$5.05 \pm 0.74ab$	$4.26 \pm 0.40b$	0.008
SDW	g	3.93 ± 0.72	4.42 ± 0.69	3.92 ± 0.27	4.92 ± 0.59	3.62 ± 0.48	3.94 ± 0.47	0.621
LA	cm ²	$972 \pm 133a$	$978 \pm 95a$	$666 \pm 117ab$	$990 \pm 147a$	$797 \pm 114ab$	$461 \pm 58b$	0.008
SLA	cm ² .g ⁻¹	$219.3 \pm 25.8a$	$177.1 \pm 7.2ab$	$87.7 \pm 14.3c$	$145.4 \pm 17.0bc$	$150.6 \pm 17.5abc$	$102.2 \pm 10.7c$	0.001
LAR	cm ² .g ⁻¹	$82.7 \pm 8.9a$	$66.5 \pm 5.7ab$	$36.4 \pm 5.1c$	$55.4 \pm 6.4bc$	$58.3 \pm 7.3abc$	$35.4 \pm 4.2c$	0.001
DW_{TR}	g	$1.88 \pm 0.26c$	$2.14 \pm 0.31bc$	$3.05 \pm 0.45abc$	$3.23 \pm 0.31ab$	$3.64 \pm 0.41ab$	$3.79 \pm 0.53a$	0.001
DW_{FR}	g	$1.60 \pm 0.37ab$	$3.47 \pm 0.91a$	$2.65 \pm 0.38a$	$2.36 \pm 0.64ab$	$0.92 \pm 0.17b$	$0.53 \pm 0.16b$	0.001
DW_{FR}/DW_{TR}	g.g ⁻¹	$0.80 \pm 0.11a$	$1.50 \pm 0.31a$	$0.98 \pm 0.17a$	$0.68 \pm 0.15a$	$0.25 \pm 0.10b$	$0.14 \pm 0.08b$	0.001
New_ DW_R	g	$3.18 \pm 0.50a$	$2.81 \pm 0.79ab$	$0.26 \pm 0.04d$	$4.74 \pm 0.83a$	$0.99 \pm 0.22bc$	$0.41 \pm 0.13cd$	0.001
DW_R/DW_s	g.g ⁻¹	0.45 ± 0.06	0.41 ± 0.14	0.51 ± 0.08	0.50 ± 0.06	0.56 ± 0.05	0.52 ± 0.05	0.818

Table 3. Root growth capacity (RGC) test.. Results for root morphology variables from one-way ANOVA (Mean \pm standard error, N = 7; Tukey's HSD *post-hoc* test). The p values in bold indicate significant differences at $p < 0.05$. Abbreviations: maximum root depth (Max_depth), root length (RL), surface area (SA), root biomass (RB), Specific root length (SRL), *Cedrela montana* (Cm), *Alnus acuminata* (Aa), *Podocarpus sprucei* (Ps), *Aegiphila ferruginea* (Af), *Schinus molle* (Sm) and *Caesalpinia spinosa* (Cs).

Root morphology		Species						P value
		<i>Cm</i>	<i>Aa</i>	<i>Ps</i>	<i>Af</i>	<i>Sm</i>	<i>Cs</i>	
Max_depth	m	0.32 \pm 0.03b	0.27 \pm 0.02b	0.13 \pm 0.03c	0.32 \pm 0.02b	0.50 \pm 0.03a	0.28 \pm 0.04b	0.001
RB	g	0.468 \pm 0.082ab	0.207 \pm 0.063bc	0.053 \pm 0.016cd	0.752 \pm 0.107a	0.612 \pm 0.064a	0.037 \pm 0.011d	0.001
RL	m	3.06 \pm 0.28c	3.49 \pm 1.05bc	1.10 \pm 0.28c	6.11 \pm 0.60ab	8.47 \pm 0.1a	0.99 \pm 0.30c	0.001
SA	cm ²	312.7 \pm 45.8b	140.3 \pm 44.7c	37.6 \pm 11.0c	495.6 \pm 48.2a	372.8 \pm 34.3ab	22.9 \pm 5.1c	0.001
SRL	m.g ⁻¹	7.72 \pm 1.29c	20.36 \pm 3.99ab	23.05 \pm 2.21ab	8.83 \pm 1.02c	14.78 \pm 2.37bc	26.09 \pm 3.17a	0.001